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Abstracts

Intracellular Signaling Pathways

306

The role of O-GlcNAc in zebrafish embryogenesis

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The role of post-translational modifications such as phosphorylation and proteolysis in regulating the activity of developmental pathways has been studied extensively. Little is known about the role of the common post-translational modification O-linked β-*N*-acetylglucosamine (O-GlcNAc). O-GlcNAc occurs on serine/threonine residues of nucleocytoplasmic proteins such as cytoskeletal proteins, tumor suppressors and transcription factors. Although studied extensively in plants and mammalian cell culture, the role of O-GlcNAc in animal development is not fully understood. The addition and removal of this modification occurs through the highly conserved enzymes O-GlcNAc Transferase (OGT) and O-GlcNAcase, respectively. We are examining the role of O-GlcNAc in vertebrate development. We have identified and cloned two ogt genes in zebrafish and will present their spatiotemporal expression pattern. Overexpressing ogt leads to a wide range of developmental defects, which we are currently characterizing. We will determine whether O-GlcNAc controls cell fate decisions or if it is required for cell viability.

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307

Wnt and G protein signaling in primitive and parietal endoderm differentiation

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Mouse F9 cells differentiate into primitive endoderm when treated with retinoic acid (RA) and into parietal endoderm when treated with RA and dibuturyl cAMP. Differentiation, marked by changes in morphology and cellular physiology, depends on numerous signals including those imparted by the Wnts. Wnt6 transfected into F9 cells causes ^{2}-catenin translocation to the nucleus and the formation of primitive endoderm. Constitutive activation of G ± 13 also causes the ^{2}-catenin translocation, however, cells differentiate through primitive to parietal endoderm. We hypothesize that differentiation to primitive endoderm requires Wnt signaling, and a G-protein-dependent pathway involving G ± 13 is necessary for differentiation to parietal endoderm. To test this, we transfected a constitutively active G ± 13 mutant into F9 cells and found that, during differentiation, it signals through p115RhoGEF and RhoA. Inhibiting Rho Kinase blocks the G ± 13, but not the Wnt6-mediated differentiation to primitive endoderm. G ± 13, known to signal through RhoA to regulate the actin cytoskeleton, can activate members of the Band 4.1 superfamily of proteins that include ezrin, radixin and moesin (ERM). Knocking down the expression of moesin in F9 cells, by shRNA or a morpholino strategy, alters the distribution of actin and ERM proteins, which leads to apoptosis. The presence of the constitutively active G ± 13 mutant in the moesin-depleted cells prevents apoptosis, but does not facilitate differentiation. Together, results indicate that Wnt6 signaling is sufficient to promote primitive endoderm formation, but G ± 13 activation is necessary to induce parietal endoderm.

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308

Signaling via GSK-3 is required during midline skeletogenesis

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GSK-3 interacts with a many pathways important for skeletal development, including Wnt, insulin and NFAT-dependent signaling. To study the roles of GSK-3 in these diverse signaling pathways, we have developed knock-in mice that allow us to specifically manipulate the stability and subcellular localization of endogenous GSK-3beta during embryogenesis. Using two different mutant alleles of GSK-3beta, a conventional knock-out and our drug-dependent allele, we find that GSK-3beta deficient mice die perinatally with a complete cleft of the secondary palate. In GSK-3beta deficient mice, the posterior frontal (PF) suture remains widely patent at birth, possibly due to a delay in suture formation. Both the palate and the PF suture are derived from the neural crest, suggesting a requirement for GSK-3 in neural-crest-dependent craniofacial development. GSK-3beta mutants also display impaired sternal fusion and delayed ossification of the sternum. Collectively, these defects are symptomatic of dysregulated Wnt and insulin signaling. While these phenotypes point to specific non-redundant roles for GSK-3beta in certain aspects of skeletogenesis, GSK-3alpha has significantly overlapping expression patterns with GSK-3beta and may exert interchangeable functions on other aspects of embryogen-

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